followed by successful engraftment. Grade 2 nausea and grade 1 emesis were seen briefly on day 2 of TMI. Skin erythema, oral mucositis, esophagitis, and enteritis were not observed. **Conclusions:** This report demonstrates the feasibility to selectively deliver myeloablative doses of radiation to bone and marrow using Tomotherapy. Organ doses were substantially lower than those associated with standard TBI and predict for the potential to significantly reduce associated toxicities, allowing for dose escalation. Ongoing trials will define the maximum TMI/TMLI doses achievable and define the potential advantages and limitations of this new approach for patients undergoing HSCT (Table1).

*Median Organ Doses (Gy) for TMI 12 and 20 Gy vs Standard TBI 12 Gy in a 20 Year Old Patient with AML*

| Organ | TMI 12 Gy | TMI 20 Gy | Standard TBI 12 Gy |
|---|---|---|---|
| **Lungs** | **4.3** | **6.8** | **8.8** |
| **Esophagus** | **3.9** | **5.6** | **12.4** |
| **Liver** | **6.0** | **8.7** | **12.3** |
| **Kidneys** | **5.6** | **8.7** | **12.2** |
| **Bowel** | **3.5** | **5.0** | **12.3** |
| **Bladder** | **7.0** | **7.4** | **12.4** |
| **Eyes** | **6.6** | **7.0** | **11.3** |
| **Parotids** | **3.9** | **4.8** | **11.8** |
| **Oral cavity** | **2.2** | **3.0** | **11.8** |
| **Stomach** | **3.1** | **5.0** | **12.2** |
| **Ovaries** | **4.3** | **6.0** | **12.3** |
| **Breasts** | **6.9** | **8.7** | **11.5** |
| **Heart** | **6.2** | **6.4** | **12.1** |
| **Thyroid** | **3.7** | **4.9** | **12.1** |
| **Brain** | **4.0** | **7.9** | **12.0** |
| **Lens** | **1.5** | **1.9** | **11.3** |

## 259

### ABSOLUTE NUMBER OF TRANSPLANTED CD34$^+$ CELLS EXPRESSING C-MPL (CD110) CORRELATES WITH SPEED OF PLATELET ENGRAFTMENT FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANTATION

*Sartor, M.M.[1], Antonenas, V.[2], Garvin, F.[2], Bradstock, K.F.[2], Gottlieb, D.J.[2] 1. Flow Cytometry Unit, Westmead Hospital., Sydney, NSW, Australia; 2. Sydney Cellular Therapies Laboratory, Westmead Hospital, Sydney, NSW, Australia.*

Recovery of neutrophil numbers after peripheral blood stem cell transplantation (PBSCT) is closely associated with graft CD34$^+$ cell dose. Predicting the speed of platelet recovery is more difficult but would be of value given that a significant minority of patients experience delayed platelet recovery and bleeding complications after transplantation. In this study we retrospectively analysed the graft composition of 29 patients who underwent autologous transplantation, using blood stem cells mobilized with cyclophoshamide and G-CSF, to assess the utility of c-mpl expression on CD34$^+$ cells as a predictor of platelet engraftment (ie, time to platelet count greater than $20 \times 10^9$/L for 3 consecutive days without the need for platelet transfusion). Absolute CD34$^+$ cells and CD34 subsets expressing c-mpl were enumerated using a published single platform viable CD34 flow cytometry assay (BMT, 2005). Of the 29 patients 7 required at least 21 days for platelet engraftment. These patients received a median graft dose of $5.7 \times 10^4$ CD34$^+$CD110$^+$ cells/kg compared with a median dose of $13.4 \times 10^4$ cells/kg received by patients who experienced platelet engraftment within 21 days of transplant ($P = .013$). In contrast, there was no difference in the number of CD34$^+$ cells/kg infused (4.0 v $4.9 \times 10^6$/kg for $>$ or $<$ 21 days for platelet engraftment respectively, $P = .23$). There was a poor correlation between the absolute number of CD34$^+$ cells and the number of CD34$^+$CD110$^+$ cells in the graft ($r^2 = 0.48$). Similarly there was no correlation between the percentage of CD34$^+$ cells expressing c-mpl and the speed of platelet engraftment (8.1 v 5.8%) for $>$ or $<$ 21 days for platelet engraftment respectively, $P = .39$). Patients with $>$21 days for platelet engraftment received platelet transfusions more often than those with $<$21 days for platelet engraftment (median 9 v 2 transfusions, $P < .001$). The absolute number of CD34$^+$/CD110$^+$ cells/kg infused at time of transplantation appears to be an important factor identifying patients at risk of delayed ($>$21 days) platelet engraftment. Those with $<6 \times 10^4$CD34$^+$/CD110$^+$cells/kg are at particularly high risk of delayed platelet engraftment requiring multiple transfusion after transplantation.

## 260

### AMD3100 + G-CSF IMPROVES HEMATOPOIETIC PROGENITOR CELL (HPC) COLLECTION IN PATIENTS WITH HODGKIN'S DISEASE (HD)

*Cashen, A.F.[1], Devine, S.[1], Vij, R.[1], DiPersio, J.[1] Washington University School of Medicine, St. Louis, MO.*

For patients undergoing autologous stem cell transplantation, the number of CD34+ cells infused is a reliable predictor of neutrophil and platelet engraftment, with doses $\geq 5 \times 10^6$ CD34+ cells/kg associated with faster count recovery. However, among the 98 patients with HD who have undergone G-CSF-alone mobilization at our institution in the past 5 years, 22% did not achieve a minimum HPC collection of $2 \times 10^6$ CD34+ cells/kg in $\leq$5 apheresis procedures, and only 15% achieved a collection $\geq 5 \times 10^6$ CD34+ cells/kg. AMD3100 mobilizes HPCs by reversibly inhibiting the interaction of CXCR4 and SDF-1$\alpha$. It has been shown to improve HPC mobilization in patients with multiple myeloma and non-Hodgkin's lymphoma. Here we present results for the first ten HD patients treated with a mobilization regimen of AMD3100 + G-CSF. Ten patients with relapsed (8) or refractory (2) HD were mobilized with G-CSF (10 ug/kg/day) + AMD3100 (240 ug/kg/day) beginning on day 4. Apheresis was performed 11 hours after each AMD3100 dose. The first dose of AMD3100 produced a median (range) 3.0 (1.9–5.1)-fold increase in the number of circulating CD34+ cells. Six patients achieved a collection of $\geq 5 \times 10^6$ CD34+ cells/kg, and all patients collected $>2 \times 10^6$ CD34+ cells/kg (range, 3.6–9.4). The median (range) number of apheresis procedures performed per patient was 2 (1–4). No grade II-IV adverse events were ascribed to AMD3100. All patients have been transplanted with G-CSF + AMD3100 mobilized cells. They have had prompt and stable engraftment, with median neutrophil recovery at day +9 (9–11) and median platelet recovery at day +16 (12–23). We conclude that AMD3100 + G-CSF is a well-tolerated and effective mobilization regimen in patients with HD. All patients (100%, 95% CI 69%–100%) mobilized with AMD3100 + G-CSF achieved the minimum collection of $2 \times 10^6$ CD34+ cells/kg, and a significantly higher proportion of patients (60%, 95% CI 26%–88%) achieved the goal collection of $\geq 5 \times 10^6$ CD34+ cells/kg than did the historical controls (15%). Importantly, the median collection in the first two days of pheresis was $5.4 \times 10^6$ CD34+ cells/kg, which is significantly better than historical controls, who collected a median $3.0 \times 10^6$ CD34+ cells/kg in the first two days of pheresis ($P = .014$). Our results demonstrate that the mobilization regimen of AMD3100 + G-CSF can improve the number of HPCs collected and decrease the number of days of pheresis in HD patients.

## 261

### HEMATOPOIETIC AC133+ STEM CELL THERAPY FOR PATIENTS WITH SEVERE PERIPHERAL VASCULAR DISEASE

*Statkute, L.[1], Oyama, Y.[1], Pearce, W.[1], Yaung, K.[1], Villa, M.[1], Shook, T.[1], Clifton, R.[1], Verda, L.[1], Krosnjar, N.[1], Burt, R.K.[1] Division of Immunotherapy, Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL.*

Hematopoietic stem cells, especially more primitive endothelial cell precursors, AC133+ cells, may contribute to neo-angiogenesis. Here we report short-term outcomes of the first 5 patients who underwent autologous selected AC133+ stem cell percutaneous transplant for their medically refractory and not-amenable to surgical reconstruction lower extremity (LE) peripheral vascular disease (PVD). Patients were 3 females and 2 males; median age was 59 (range 26–84) years old. Peripheral blood AC133+ cells were mobilized with G-CSF 10 mcg/kg/day for 4 days. Median apheresis number of was 1 (range 1 to 2). AC133+ cells were enriched using CliniMACS device, Miltenyi Biotec, Inc. Total number of

injected AC133+ stem cells in 4 patients was 38.2, 141, 170, 51.3 million, and 28 million of CD34+ cells in 1 patient. Three patients underwent percutaneous intramuscular AC133+ stem cell transplant into the right LE, 2 patients into the left LE. Injection sites were marked by ultrasound and were 4 to 20 sites per extremity. There were no complications associated with the procedure. Median follow-up after the transplant is 4 (range 1.5–11) months. First patient with very advanced LE ischemia, unfortunately, was involved into study too late as ischemia continued to progress and eventually patient required a below-knee amputation. Second patient with Burger's disease, now 7.5 months after the transplant, has had significant improvement in symptoms such as rest pain and claudication. Objective testing at 3- and 6-month follow-up showed improvement in all parameters, including detectable new faint posterior tibialis (PT) artery pulse, improved four-meter and six-minute walk tests, walking impairment questionnaire, Gardner graded treadmill test, and Doppler arterial brachial index, dorsalis pedis (DP), and PT values increasing from 0.34, 28, 37 to 0.37, 37, 43, respectively. Additionally, MRA of LE showed increasing number of collateral vessels in a diseased calf. Last 3 patients had a short follow-up with no objective testing repeated, although all of them reported marked symptom improvement and increased walking capacity. In last 2 patients, we detected faint DP and PT pulses, which were absent before procedure. Our preliminary data suggest that AC133+ stem cell percutaneous transplant can be performed safely and appears to be beneficial therapy for selected patients with severe peripheral vascular disease.

## 262

### LEUKOCYTE AND PLATELET COUNTS CAN BE USED TO GUIDE COLLECTION OF AUTOLOGOUS STEM CELLS AFTER G-CSF MOBILIZATION ON THE FIRST TWO DAYS OF LEUKAPHERESIS BUT NOT BEYOND

*Gidron, A.[1], Mehta, J.[1] Division of Hematology/Oncology, Northwestern University, Chicago, IL.*

When donors are treated with G-CSF to collect stem cells for allogeneic transplantation, the combination of a pre-apheresis leukocyte count of $\geq 25 \times 10^9$/L **and** a pre-apheresis platelet count of $\geq 100 \times 10^9$/L is associated with excellent mobilization as measured by an absolute peripheral blood CD34+ cell count (PBCD34) of 20/μL, and can be used to guide harvest timing (Tomblyn et al Bone Marrow Transplantation advance online publication 01 August 2005; doi: 10.1038/sj.bmt.1705117). We wanted to see if this observation could be extended to patients receiving 10–16 μg/kg G-CSF daily as the sole mobilizing agent for autologous stem cell collection. Data from 161 leukapheresis procedures (65 day 1 collections, 47 day 2 collections, and 49 collections done beyond day 2) were analyzed to determine correlation between pre-apheresis leukocytes, platelets, and PBCD34. Overall, when leukocytes ≥25 and platelets ≥100, PBCD34 was 2.9–515.5 (median 31.8); significantly ($P < .0001$) higher than PBCD34 of 0.6–226.0 (median 6.0) when leukocytes were <25 and/or platelets were <100. Among day 1 collections, with leukocytes ≥25 and platelets ≥100, PBCD34 was 20 and 10 in 63% and 78% of the time compared to 16% and 36% of the time if leukocytes were <25 and/or platelets were <100 ($P = .0001$ and $P = .001$, respectively). Similarly, PBCD34 levels of 20 and 10 were obtained 63% and 87% of the time with day 2 collections when leukocytes were ≥25 and platelets ≥100, compared with 19% and 35% of the time when leukocytes were <25 and/or platelets were <100 ($P = .003$ and 0.001, respectively). Beyond day 2, when leukocytes ≥25 and platelets ≥100, PBCD34 was 20 50% of the time compared with 7% when leukocytes were <25 and/or platelets were <100 ($P = .047$). Our data suggest that in patients with hematologic malignancies receiving G-CSF for mobilization of autologous stem cells, the combination of a leukocyte count of ≥25 and a platelet count of μ100 is associated with excellent mobilization of stem cells in the majority of patients on the first and second harvest days but not beyond. Conversely, lower leukocyte and/or platelet counts predict for poor mobilization beyond the second day, but not on the first two days. Thus, on the first and second days of stem cell collection in patients mobilized with G-CSF, apheresis can be initiated based on favorable hematologic parameters without necessarily awaiting PBCD34 levels. However, beyond the second day, PBCD34 levels should guide apheresis.

## 263

### SYNGENEIC NEUROBLASTOMA CELLS TRANSIENTLY-TRANSFECTED WITH PLASMID DNA VECTORS ENCODING A PANEL OF IMMUNE STIMULATORY MOLECULES CAN INDUCE ANTI-TUMOR IMMUNITY EARLY AFTER BMT

*Jing, W.[1], Yan, X.[1], Orentas, R.J.[1], Johnson, B.D.[1] Medical College of Wisconsin, Milwaukee, WI.*

Mouse neuroblastoma (Neuro-2a) cells genetically modified to express CD54 (ICAM-1), CD80, CD86, and CD137L (4-1BB ligand) can serve as a tumor vaccine. The goals of this study were to determine the cellular components required to induce tumor-protective immunity with this vaccine early after syngeneic bone marrow transplantation (BMT), and to determine if the cell-based vaccine could prevent tumor growth in a minimal residual disease model. Lethally irradiated A/J mice were transplanted with syngeneic BM cells with or without purified T cells or splenocytes (as a source of T cells). The Neuro-2a-based vaccine was given to transplant recipients on days 7 and 14 post-transplant. Vaccination of mice given BM without added T cells failed to protect mice from a challenge of $10^5$ or $10^6$ viable tumor cells. In contrast, 79% of vaccinated mice given T cells at the time of BMT survived tumor challenge. Development of protective immunity was dependent upon both $CD4^+$ and $CD8^+$ T cells. We concluded that the adoptive transfer of T cells was absolutely required for induction of tumor vaccine-induced protective immunity. We examined whether depletion of $CD25^+$ regulatory T cells from the adoptively-transferred T cells or in vivo CD25 depletion of the transplant recipients would further increase vaccine-induced tumor immunity, but they did not. This suggests that the lethal conditioning itself had eliminated the majority of $CD25^+$ regulatory T cells. To more closely mimic treatment of "minimal" residual disease, A/J mice transplanted with BM and splenocytes were inoculated with $10^4$ AGN2a cells one day after BMT. Tumor vaccination was administered on days 2 and 9 post-BMT. Tumor vaccination in this model failed to protect mice from tumor progression. However, in a preliminary experiment, post-BMT vaccination with tumor cells expressing CD54, CD80, CD86, CD137L, and IL-15 could prevent tumor progression if splenocytes given at the time of BMT were from tumor-primed donors. Collectively, these results demonstrate that anti-neuroblastoma immunity can be induced early after BMT using novel cell-based cancer vaccines; however, in a setting of minimal residual disease, a combination of adoptive immunotherapy and tumor vaccination is required to prevent post-transplant tumor relapse.

## 264

### MITOXANTRONE AND MELPHALAN AS A CONDITIONING REGIMEN FOR AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN ADULTS WITH ACUTE MYELOGENOUS LEUKEMIA

*Sprague, K.A.[1], Padgaonkar, V.A.[1], Klein, A.K.[1], Chan, G.W.[1], Miller, K.B.[2], Klingemann, H.[1] 1. Tufts-New England Medical Center, Boston, MA; 2. Beth Israel-Deaconess Medical Center, Boston, MA.*

A variety of conditioning regimens are used for autologous hematopoietic stem cell transplantation in patients with acute myelogenous leukemia (AML). High dose mitoxantrone and melphalan (Mito/Mel) has been reported as an effective and well-tolerated regimen for autologous peripheral blood stem cell transplantation (APBSCT) in patients with lymphoma. We review our experience with Mito/Mel as a conditioning regimen in 18 adult patients (12 female, 6 male) with intermediate or high risk AML who underwent APBSCT. All patients received anthracycline based induction and consolidation chemotherapy prior to APBSCT. At the time of transplantation, 16 patients were in first complete remission (CR) and 2 patients were in second CR. Median age at the time of APBSCT was 51 years (range 19–70). All patients received a conditioning regimen consisting of mitoxantrone 60 mg/m2 IV on day −4 and melphalan 180 mg/m2 IV on day −1, followed by infusion of autologous peripheral blood stem cells on day 0. Rapid